

Recent Studies in Surface Disinfection

By R. L. STEDMAN, Sc.D., and E. KRAVITZ, Sc.D.

ALTHOUGH many studies on disinfection have appeared in the literature during the past 75 years, significant and unexpected gaps in our knowledge of germicides remain. Efficiency in disinfecting floors, walls, and ceilings is one of the more significant gaps requiring detailed study. Until 1953, little information on the basic aspects of such disinfecting operations was available although Varley and Reddish (1) and Klarmann and associates (2) presented important but limited data.

For the past few years, the Department of the Navy has been conducting an extensive investigation of the problem at the Industrial Test Laboratory under the cognizance of the Bureau of Ships and Bureau of Medicine and Surgery. Because of the extent of the work, certain portions of this investigation were performed under contract at the Bacteriological Unit, Plant Pest Control Branch, Agricultural Research Service, Department of Agriculture, under the direction of Dr. L. S. Stuart. The salient findings obtained from these investigations to date and the results of certain other pertinent studies recently reported are reviewed in the current report.

When critically examined, disinfection of floors, walls, and ceilings represents a comparatively complex problem because of the many variables encountered in diverse disinfecting

operations. Such variables as the composition of the surface, the degrees of soil and microbial contamination, the type of micro-organisms present, the use of a washing procedure before disinfection, the degree of hardness of the water used in preparing the disinfectant dilutions, and many other factors represent a gamut of test conditions to be examined in a study of this nature.

Initially, it was obvious that not all of these conditions could be thoroughly investigated and that some compromise was required. Ultimately, it was decided to limit the study to floor disinfection by simulated use methods incorporating the various conditions described below.

Principal Test Methods

Two basic procedures for determining antimicrobial activity were developed for most of the work reported here (3-5). The first, the "Stuart" procedure, was used to measure the effect of precleaning on subsequent disinfection and consists of successively contaminating, cleaning, and disinfecting a large square of surface material. The pattern of elimination of micro-organisms is followed throughout the simulated precleaning and disinfection. The second, the "Square-Diluent" procedure, consists of contaminating and disinfecting 1-inch squares of surface materials in a manner that simulates actual conditions. This technique was used in all studies in which surfaces were not precleaned before disinfection.

The first technique gives a valid picture of the relative changes in antimicrobial numbers since a swab recovery technique is employed. The second method is of more value when the

Dr. Stedman is head, Bacteriological Group, and Dr. Kravitz is supervisory bacteriologist with the Industrial Test Laboratory, Philadelphia Naval Shipyard, Department of the Navy. The opinions expressed are not necessarily the views of the Department of the Navy.

absolute numbers of microbial survivors are required. Both techniques are versatile and permit the inclusion of many significant variables in actual disinfecting procedures.

Disinfectant Specificity

Obviously, the most useful disinfectants possess a minimum of antimicrobial specificity. In practical terms, this means that a disinfectant should be effective against a wide spectrum of microbial species when employed at a practical use-dilution. The particular specificities of some types of germicides have been known for some time, for example, the failure of unfortified pine oil formulations to be effective against pyogenic cocci. However, deficiencies in other types became apparent during the study. Table 1 presents representative data to illustrate this point.

On surfaces not precleaned, the quaternary ammonium germicides are effective under certain conditions. Substantial increases in the manufacturers' recommended concentrations of quaternaries are needed to achieve a high degree of bactericidal activity, but the new use-dilutions are not impractical to employ (table 1). However, these germicides are seriously deficient in antifungal activity, and require impractically strong concentrations in most instances.

The particular chlorine product tested was

much less effective against pyogenic cocci than against enteric bacilli and dermatophytic fungi (table 1). However, it cannot be stated at this time that chlorine products in general show this extreme specificity under conditions simulating floor disinfection because of the limited number of chlorine products tested. Also, it should be emphasized that such specificity may not be evident when the above or any chlorine product is employed as a sanitizer or water decontaminant; the environmental conditions in these instances, bacterial load, exposure time, physicochemical factors, and the like, are entirely different from those encountered in floor disinfection.

The synthetic phenolic and unfortified cresylic acids and coal tar products show less specificity than the other products. In most instances, the manufacturer's recommended use-dilutions are effective against the three test organisms. This is, perhaps, understandable, since the classical determination of use-dilution by extrapolation of laboratory data, that is, use-dilution in practice = $20 \times$ phenol coefficient obtained in a standard laboratory procedure, has been found to be more applicable to synthetic phenolics and related types than to quaternary ammonium germicides and halogens (6). However, discrepancies can readily be demonstrated even with the phenolics (7). This entire question of the correlation between phenol coefficient and recommended use-dilution is of sig-

Table 1. Disinfection of a nonporous surface (stainless steel) by various germicides without precleaning

| Germicide | Recom- mended use- dilution | Effective dilutions ¹ | | | | | |
|--------------------------|--------------------------------------|----------------------------------|--------|--------|------------|--------|--------|
| | | Without serum | | | With serum | | |
| | | MPA | SS | TI | MPA | SS | TI |
| Phenolic A..... | 1:250 | 1:250 | 1:1500 | 1:250 | 1:130 | 1:250 | 1:130 |
| Cresylic..... | 1:150 | 1:150 | 1:600 | 1:180 | 1:150 | 1:600 | 1:150 |
| Chlorine..... | ² 1:5000 | 1:310 | 1:3100 | 1:4600 | <1:310 | 1:3100 | 1:4000 |
| Quaternary ammonium..... | 1:2500 | 1:1000 | 1:1000 | <1:100 | 1:1000 | 1:1000 | <1:100 |

¹ Dilutions of formulations (active ingredients only) required to obtain 99.99 percent reduction (bacteria) or 99.9 percent reduction (fungus) in Square-Diluent method. See reference 4 for details.

² Based on available chlorine.

MPA = *Micrococcus pyogenes* var. *aureus*; SS = *Salmonella schottmuelleri*; TI = *Trichophyton interdigitale*.

nificance in the concept of disinfection but has been adequately treated elsewhere (6, 7).

Surface Porosity

Although the above generalization on the relative effectiveness of disinfectant types is valid, surface porosity tends to alter the quantitative pattern in certain instances (table 2). For example, certain phenolic formulations lose much more activity than others in changing from a nonporous to a porous surface (8).

Table 2. Effect of surface porosity in reducing antimicrobial efficiency of disinfectants

| Germicide | Bactericidal activity ¹ | | | (B) (A) | (C) (A) |
|-------------------------------|------------------------------------|---------------------|----------------------------|------------|------------|
| | Stainless steel (A) | Asphalt tile (B) | Battleship linoleum (C) | | |
| Phenolic A..... | 1:50 | 1:25 | 1:10 | 0.50 | 0.20 |
| Phenolic B..... | 1:100 | 1:5 | 1:5 | 0.050 | 0.050 |
| Phenolic C..... | 1:125 | 1:25 | ----- | 0.20 | ----- |
| Phenolic D ₁ | 1:100 | 1:25 | ----- | 0.25 | ----- |
| Phenolic D ₂ | 1:200 | 1:50 | ----- | 0.25 | ----- |
| Cresylic..... | 1:100 | 1:100 | 1:10 | 1.0 | 0.10 |
| Coal tar..... | 1:120 | 1:10 | ----- | 0.083 | ----- |

¹ Dilutions of formulation required to reduce *Micrococcus pyogenes* var. *aureus* to 99.99 percent of original number in the presence of serum.

SOURCE: Reference 8.

The extent of this loss is apparently determined by the nature of the porous surface since significant differences in activity are observed on materials such as asphalt tile, battleship linoleum, soapstone, and wood. In most instances, chemical interaction between surface and germicidal agent is not observed and it seems valid to infer that porosity per se accounts in a large measure for these differences.

Superficial observation of the effective dilutions of germicides required for disinfection of the various porous surfaces (table 2) shows that impractically high concentrations are needed in many instances. Also, a different use-dilution of the same disinfectant may be

required for each porous surface. Obviously, it is not practical to employ such a multiplicity of use-dilutions with a disinfectant product. Some alternative procedure must be used to combine antimicrobial effectiveness and simplicity of operation.

Studies on this point have revealed that the effectiveness of the use-dilution recommended for a nonporous surface can be enhanced on a porous surface by the use of long exposure times and by successive treatments of the surface with germicide (9, 10).

As might be expected, the reduction of microbial numbers increases with length of exposure time and continues even after drying of the disinfectant is visibly completed. However, the time relationship is not linear, and after the first 10 minutes of exposure, the survivor curves tend to become asymptotic. For all practical purposes, the effective reduction in numbers is reached after the first 30 minutes.

Two successive applications of disinfectant are more effective than a single prolonged application in most instances, although the same pattern of initial rapid action followed by an asymptotic rate of reduction is encountered. With some disinfectants it appears that many successive applications on porous surfaces are required to achieve the same degree of effectiveness as attained on a nonporous surface such as stainless steel. At any rate, a significant increase in efficacy can be obtained by the use of long exposure times and successive germicidal applications, thus permitting a single use-dilution of disinfectant to be employed on a wide variety of surfaces.

Cumulative Effect

A daily routine program of applying disinfectants to floors produces an enhanced sanitary effect. Apparently, each successive daily application provides a prolonged residual of disinfectant which contributes to the antimicrobial efficiency of the next application (9). The degree of contribution is undoubtedly a function of the rate of evaporation, that is, vapor pressure, of the particular product since formulations vary rather widely in this respect. Ambient relative humidity is also of significance

Table 3. Physicochemical properties of formulations displaying various degrees of retention of bactericidal activity on changing from a nonporous to a porous surface

| Germicide | Comparative order of efficiency ¹ | | | |
|-------------------------------|--|--------------------------------|-------------------------|---|
| | Surface tension depression ² | Spreading wetting ² | Detergency ² | Retention of bactericidal activity ³ |
| Cresylic----- | 2 | 1 | 2 | 1 |
| Coal tar----- | 6 | 6 | 6 | 6 |
| Phenolic A----- | 4 | 5 | 3 | 2 |
| Phenolic B----- | 7 | 7 | 7 | 7 |
| Phenolic C----- | 5 | 4 | 1 | 5 |
| Phenolic D ₁ ----- | 1 | ⁴ 2-3 | ⁴ 4-5 | ⁴ 3-4 |
| Phenolic D ₂ ----- | 3 | ⁴ 2-3 | ⁴ 4-5 | ⁴ 3-4 |

¹ Relative effectiveness of the seven germicides for each of the indicated properties. 1=most effective; 7=least effective.

² See reference 12 for techniques and details.

³ Based on ratio of bactericidal activities on porous and nonporous surfaces. See table 2.

⁴ These products gave identical results in the indicated tests.

in this regard (11, 12). With many disinfectants the residual is sufficient to kill small numbers of organisms without the aid of additional disinfectant after contamination (2, 9, 11). Such an effect may be of importance in the elimination of dustborne hemolytic streptococci and other organisms in hospital wards, dispensaries, and the like.

Formulation Properties

As noted above, disinfectant formulations, even of the same chemical type, vary widely in the degree of antimicrobial activity retained on a nonporous as compared to a porous surface.

These variations in retention of activity have been shown to be due, at least in part, to differences in certain physicochemical properties of formulations (12): surface tension depression, spreading wetting and detergency. Although a quantitative correlation between retention of activity and any of these properties could not be demonstrated, some relationship was noted (table 3).

Products with poor or excellent retention were found to possess relatively poor or excellent efficiencies in the physicochemical proper-

ties. This relationship is, perhaps, not unexpected since surface tension depression, wetting, detergency, suspending power, emulsification, and other similar properties contribute in various degrees to the penetration and cleansing of porous surfaces. Superior penetration and disinfection of the crevices and pores of surfaces such as battleship linoleum or asphalt tile would be anticipated with a product outstanding in the above physicochemical properties. Undoubtedly, the failure to establish a concise correlation shows a complex interrelation of the many physicochemical properties which contribute to disinfection.

Ortenzio and associates (13) have also emphasized the importance of the physicochemical properties of formulations in disinfection. A graphic demonstration of the enhancement of disinfectant efficiency was shown by the addition of small amounts of cleaners and sequestering agents to various types of disinfectant use-dilutions. The enhancement was believed to have resulted from an improvement in the soil suspending and dispersing properties of the solution. The authors concluded that consideration should be given to requiring certain standards for soil suspending and dispersing properties of disinfectants when a combined cleaning and disinfecting action is claimed on the label.

Unfortunately, many commonly employed laboratory methods for determining disinfectant activity present an array of physicochemical factors which bear little or no relationship to those encountered when the disinfectant is used in practice, although a tendency has been noted more recently to employ simulated use procedures. Further effort should be devoted to a study of such procedures and to the development of formulations having physicochemical properties which enhance antimicrobial effectiveness in use.

Precleaning

On superficial examination, it might be expected that precleaning of a contaminated surface prior to disinfection would produce a much more effective process than disinfection without precleaning. However, there are certain obvious objections to such a process. In some in-

stances, the handling of infectious matter without disinfectant protection during manipulation of swabs and buckets while precleaning may be a potentially hazardous operation. Also, the additional work of precleaning detracts from the simplicity of the operation. The presence of cleaner residues may deleteriously affect subsequent disinfection if an intermediate rinsing step is not employed. The inclusion of such a step adds still more complexity to the operation. Other disadvantages can be detailed. Nevertheless, it seemed of significance to study a number of phases involving precleaning.

The efficiency of mechanical removal of micro-organisms from surfaces by cleaners has been shown to be a function of the porosity of the test surface. Flannery and associates (3) observed that, using standard Navy soap powder, dried white oak was more difficult to decontaminate than soapstone; stainless steel was most easily decontaminated of the three surfaces studied. No significant difference was found when four different types of cleaners, white floating soap, a non-ionic detergent, trisodium phosphate, and Navy soap powder, were tested under comparable conditions on a white oak surface.

Surprisingly large numbers of organisms

were shown to resist removal by mechanical cleaning. For example, after six successive washings of an artificially contaminated porous oak surface, approximately 2-6 percent of the original number of organisms still remained on the wood. Assuming an initial arbitrary load of 2,000,000 organisms per square inch, a relatively large number, 40,000-120,000, would still be present after precleaning in such cases. Although decontamination of stainless steel was more easily accomplished (99.90-99.98 percent of original cell numbers removed by two washings), small numbers of organisms could still be recovered from the nonporous surface even after six consecutive washings. The concentration of the cleaner affected the efficiency of removal in some instances, but the differences were not striking.

Evidently, mechanical removal of micro-organisms by precleaning does not obviate the need for a very efficient disinfectant. Further data on this point are shown in table 4. An artificially contaminated soapstone surface was precleaned (one wash) with trisodium phosphate and then disinfected with various levels of sodium hypochlorite or the quaternary ammonium germicide, alkyl (C_8H_{17} - $C_{18}H_{37}$) dimethyl benzyl ammonium chloride (3). In no case was complete elimination of all test or-

Table 4. Efficiency of halogen and quaternary ammonium disinfectants in decontaminating soapstone after one precleaning step with 0.2 percent trisodium phosphate ¹

| Dilution of disinfectant used | Sodium hypochlorite | | | | Alkyl (C_8H_{17} - $C_{18}H_{37}$) dimethyl benzyl ammonium chloride | | | |
|-------------------------------|--------------------------------------|--|-----|-----|--|--|-----|-----|
| | Percentage reduction (all organisms) | Percentage positive swabs in qualitative tests | | | Percentage reduction (all organisms) | Percentage positive swabs in qualitative tests | | |
| | | SS | SF | TI | | SS | SF | TI |
| 1:10,000 | 91.7 | 100 | 100 | 100 | 94.5 | 100 | 100 | 100 |
| 1:5,000 | 99.6 | 100 | 100 | 25 | 97.8 | 100 | 100 | 100 |
| 1:2,500 | 99.5 | 50 | 100 | 25 | 97.7 | 100 | 100 | 100 |
| 1:1,670 | 99.88 | 50 | 100 | 25 | 97.3 | 100 | 100 | 100 |
| 1:1,250 | 99.88 | 0 | 100 | 25 | 97.8 | 100 | 100 | 100 |
| 1:1,000 | 99.84 | 0 | 100 | 25 | 99.1 | 50 | 100 | 100 |

¹ 12- x 24- x 2-inch block of soapstone contaminated with mixture of three test organisms and soil. Surface washed once with 0.2 percent trisodium phosphate, the cleaner drained off, and the area disinfected with indicated disinfectants. Standard (4" x 4") areas then swabbed, the swabs rinsed in water and then incubated in appropriate differential media. "Percentage reduction" refers to number of organisms recovered in swab rinse water. "Percentage positive swabs" refers to total number swabs which were positive after incubation. SS=*Salmonella schottmuelleri*; SF=*Streptococcus faecalis*; TI=*Trichophyton interdigitale*.

Source: Reference 3.

ganisms achieved with one application of either germicide up to concentrations of 1,000 p.p.m. after precleaning. Antimicrobial effects were initially observed at 200 p.p.m. halogen and 1,000 p.p.m. quaternary.

It was concluded: "The concentrations of germicides necessary to produce disinfection of soiled surfaces after cleaning by a swab-washing procedure (with the exception of highly polished surfaces such as stainless steel) appear to be from three to five times as great as those commonly accepted for use as final germicidal rinses for dishes and glasses in restaurants, and utensils and equipment in dairies and food plants."

The latter portion of this quotation is of significance in that hypochlorites and quaternaries are used primarily as sanitizing agents, and it was desired to relate in some fashion recommended sanitizing use-dilutions with effective disinfecting operations.

In general, the data in tables 2 and 4 (3, 8-10) indicate that the porosity of the test surface is of prime importance in deciding the effectiveness of a precleaning operation in reducing the load on the disinfectant subsequently employed. Porous surfaces can be more easily decontaminated when precleaned, but relatively strong concentrations of germicide are still required in the subsequent disinfecting operation. Unfortunately, precleaning appears to be most effective under conditions in which disinfection alone can be readily accomplished, that is, on a nonporous surface. It is questionable whether precleaning is worth the effort under such conditions, assuming that an effective disinfectant at proper concentration is ultimately employed.

Effect of Cleaner Residues

As noted above, residues from precleaning procedures may affect deleteriously subsequent disinfection if such residues are not thoroughly removed by rinsing. Ortenzio and co-workers (14) have demonstrated the extent of this inactivation using quaternary ammonium and phenolic disinfectants.

As expected, the chemical nature of the cleaner determines the extent of inactivation. Soaps are more deleterious to quaternaries than phenolics, and the reverse is true for non-ionic

detergent cleaners. When the cleaner and disinfectant are "incompatible," as much as 2.5 times more disinfectant is needed to produce the same antimicrobial effect as in the case of a "compatible" combination. Even alkaline inorganic cleaners, such as trisodium phosphate and sodium carbonate, may seriously inactivate quaternary ammonium disinfectants if soil containing fat is present, presumably due to formation of traces of soap.

It is apparent that an effective precleaning procedure, if employed, must be discriminately chosen and be properly performed.

Waxing

Since the practice of waxing surfaces is widespread in civilian and military establishments where pathogenic micro-organisms may be of significance, the effect of such a practice on subsequent disinfection has been determined (9). Surprisingly, the antimicrobial effectiveness of disinfectants on a waxed linoleum surface was not found to be strikingly different from an unwaxed surface. This was attributed to the failure of the wax to form a microscopically smooth outer layer and, in effect, transform the porous linoleum surface into a nonporous one. The waxed surface was significantly scratched and pitted after the waxing operation, presumably, because of the action of the buffing machine and solvent evaporation. For all practical purposes, the waxed surface was still porous.

Quite recently, "germicidal" floor waxes have been placed on the market. Such products consist of self-polishing water emulsion waxes with germicidal agents added (15). Present formulations list either a quaternary ammonium or a phenolic disinfectant as the biologically active ingredient. Possibly, these products act physically in a manner similar to "insecticidal" waxes which have been in use for a number of years: The biologically active material slowly leaches to the surface of the wax and provides an insecticidal or germicidal outer layer.

Since only preliminary data are available on the efficacy of germicidal floor waxes, a definitive conclusion on their usefulness cannot be made at present. However, it has been shown that these formulations are capable of eliminating small numbers of organisms which are de-

posited on wax-coated surfaces in simulated-sneezing experiments (15). Probably, moisture is required for a lethal action to occur, as in the case of all known antimicrobial agents, and ambient relative humidity may play a significant role.

The degree of effectiveness may be equivalent at best to that of disinfectant residues remaining on surfaces as a result of a routine daily treatment, but significant elimination of gross contamination deposited on surfaces coated with germicidal waxes without further addition of a disinfectant seems distinctly improbable. The exact role of these agents in environmental sanitation must await further study.

Summary and Conclusions

The salient points obtained in an extensive investigation of floor disinfection conducted by the Department of the Navy have been presented. The implications of other current findings in the literature have also been integrated and presented.

Using test procedures that attempt to simulate use conditions, investigators determined that the degree of efficiency of disinfectants used on floors is influenced markedly by the porosity of the floor surface. Certain representative chemical types of disinfectants were shown to be deficient in antimicrobial activity particularly in regard to species specificity. With synthetic phenolic formulations, the efficiency of disinfection of porous surfaces is related significantly to the physicochemical properties of the formulation. However, by long exposure times or by successive treatments with germicide, a significant reduction in microbial numbers on a porous surface can be achieved. Waxing of porous surfaces apparently does not alter strikingly the efficiency of disinfection attained on the unwaxed surface. A daily routine of disinfection contributes significantly to the ease with which porous surfaces can be decontaminated.

Studies on the effect of precleaning surfaces before disinfection have shown that the efficiency of the cleaning operation is also intimately related to surface porosity. Unfortunately, the contribution of precleaning to the

disinfecting process is greatest on nonporous surfaces which are, in themselves, relatively easy to decontaminate by a single application of germicide. The types of cleaner and disinfectant employed must be carefully chosen since residuals of cleaner remaining may seriously inactivate the germicide if the two are incompatible.

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Shellfish Sanitation Workshop

A Shellfish Sanitation Workshop, held in Washington August 27 and 28, 1956, had a registered attendance of 58. Fourteen States were represented by 18 persons. The oyster industry was represented by 6 persons designated by the Oyster Institute of North America and by 2 representatives from the National Fisheries Institute. The Canadian Government had two representatives (Department of National Health and Welfare, and Department of Fisheries). Other agencies or organizations with representatives present included the Public Health Service, Departments of Army, Navy, and Air Force, Food and Drug Administration, Fish and Wildlife Service, American Cyanamid Co., and the University of Maryland. The Association of State and Territorial Health Officers was represented by Dr. Mack I. Shanholtz of Virginia.

The manual of recommended practice for sanitary control of the shellfish industry, as revised at the meeting, was unanimously adopted by the workshop for use as a guide in the cooperative shellfish certification program.

On the basis of studies made by the Canadian Department of National Health and Welfare, the Maryland Department of Health, the Virginia Department of Health, and the Public Health Service Shellfish Sanitation Laboratory, a 1-year interim

bacteriological market standard was adopted for shucked oysters. This interim standard is the first of its kind in the 31-year history of the shellfish program and establishes three categories of evaluation:

| Category | Coliform MPN | Standard plate count |
|---------------------------------------|----------------------------------|------------------------------|
| Acceptable..... | Not more than 16,000 per 100 ml. | Not more than 50,000 per ml. |
| Acceptable on condition. ¹ | Less than 160,000 per 100 ml. | Less than 1,000,000 per ml. |
| Rejectable..... | 160,000 or more per 100 ml. | 1,000,000 or more per ml. |

¹ Shipments will be reported to the shellfish control organization of the originating State for investigation and will not be rejected unless the report of the investigating authority is unsatisfactory.

The workshop also considered effects of the dis-Public Health Service to undertake an investigation of organisms other than coliforms as indicators of the sanitary quality of shellfish.

The workshop also considered effects of the disposal of wastes from cabin cruisers and other shipping. Harold F. Udell, New York State Department of Conservation, estimated there were approximately 15 to 16 thousand pleasure craft equipped with toilet facilities and registered in the Marine District of the State of New York.